

Claims

1. Process for detecting antibiotic resistances in microorganisms, comprising the steps of:
- 5 a) preparing a sample containing microorganisms,
b) bringing the sample into contact with at least one hybridization probe, which is specific for a nucleic acid sequence in microorganisms which is associated with antibiotic resistances,
10 under conditions which permit the probe to hybridize specifically, and
c) analyzing the sample in situ by determining the appearance or absence of a hybridization.
- 15 2. Process according to Claim 1, characterized in that the microorganisms are selected from bacterial organisms and protozoa.
- 20 3. Process according to Claim 1 or 2, characterized in that the nucleic acid sequence which is associated with antibiotic resistances is selected from ribosomal nucleic acid sequences.
- 25 4. Process according to Claim 3, characterized in that the nucleic acid sequence is selected from bacterial 23 S ribosomal nucleic acid sequences.
- 30 5. Process according to Claim 4, characterized in that the nucleic acid sequence encompasses a region corresponding to one or more of the nucleotides
35 2032, 2057, 2058, 2059, 2503 and 2611 on the E.coli 23S rRNA.
6. Process according to one of the preceding claims,

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7. Process according to Claim 6, characterized in that the microorganisms are selected from the group consisting of Helicobacter spec., mycobacteria, Porphyromonas gingivalis, Propionibacterium acnes, Borrelia burgdorferi, mycoplasmas, chlamydias, Tropheryma whippelii, bartonellas, legionellas, nocardias and actinomycetes.

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- 15 8. Process according to ~~one of the preceding claims,~~
characterized in that
use is made of a sample which is derived from
human or animal tissues or body fluids.

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- 20 9. Process according to ~~one of the preceding claims,~~
characterized in that
the sample is investigated without the
microorganisms being previously cultured.

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- 25 10. Process according to ~~one of the preceding claims,~~
characterized in that
the sample is subjected to a procedure for
enriching microorganisms.

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- 30 11. Process according to ~~one of the preceding claims,~~
characterized in that
a presumptive medium is added to the sample prior
to the investigation.

- 35 12. Process according to Claim 11,
characterized in that the presumptive medium
the presumptive medium contains an indicator
substance for typing microorganisms.

[illegible]

- A 13. Process according to ~~one of the preceding claims~~,
characterized in that
the sample is fixed, and, where appropriate,
5 permeabilized, prior to the investigation.
- A 14. Process according to ~~one of the preceding claims~~,
characterized in that
the hybridization probe is selected from nucleic
10 acids such as DNA or nucleic acid analogues such
as PNA.
- A 15. Process according to ~~one of the preceding claims~~,
characterized in that
15 the hybridization probe possesses a hybridization
region having a length corresponding to from 10 to
30 nucleotide building blocks, preferably from 15
to 20 nucleotide building blocks, in particular
from 17 to 18 nucleotide building blocks.
- A 16. Process according to ~~one of the preceding claims~~,
characterized in that
20 use is made of a hybridization probe which is
specific for mutations selected from deletions,
transversions, transitions and modifications of
25 the corresponding wild type sequence.
- A 17. Process according to ~~one of the preceding claims~~,
characterized in that
30 use is made of a combination of several
hybridization probes which are specific for
different nucleic acid sequences associated with
antibiotic resistances.
- A 18. Process according to ~~either Claim 16 or 17~~,
35 characterized in that

use is made of the hybridization probes ClaR1 (SEQ ID NO. 1), ClaR2 (SEQ ID NO. 2) and/or ClaR3 (SEQ ID NO. 3).

A 19. Process according to ~~one of the preceding claims~~,
characterized in that
use is additionally made of at least one
hybridization probe which is specific for a
nucleic acid sequence which is associated with a
wild type of the microorganism.

20. Process according to Claim 19,
characterized in that
use is made of the hybridization probe ClaWT (SEQ ID NO. 4).

X 21. Process according to ~~one of the preceding claims~~,
characterized in that
use is additionally made of at least one
hybridization probe which is specific for a
species or a genus of microorganism.

22. Process according to Claim 21,
characterized in that
use is made, for detecting Helicobacter pylori, of
hybridization probes which are directed against
sequences from H.pylori 16S rRNA which are
homologous with the E.coli regions 110-140,
740-780, 585-605 and/or 210-245.

23. Process according to Claim 22,
characterized in that
use is made of the hybridization probes Hpyl-165-
753 (SEQ ID NO. 5), 120b (SEQ ID NO. 6), 585 (SEQ ID NO. 7) and/or 219 (SEQ ID NO. 8).

24. Process according to Claim 21,
characterized in that

use is made, for detecting *Helicobacter heilmannii*, of hybridization probes which are directed against sequences from *H. heilmannii* 16S rRNA which are homologous with the *E. coli* regions 580-610 and/or 640-670.

25. Process according to Claim 24, characterized in that use is made of the hybridization probes Hh1 (SEQ ID NO. 9), Hh2 (SEQ ID NO. 10), Hh3 (SEQ ID NO. 11) and/or Hh4 (SEQ ID NO. 12).
26. Process according to ~~one of the preceding claims~~, characterized in that use of made of hybridization probes which carry a direct label.
27. Process according to ~~one of the preceding claims~~, characterized in that use is made of hybridization probes which are labeled, or can be labeled, with dye groups, fluorescence groups and/or enzyme groups.
28. Process according to ~~one of the preceding claims~~, characterized in that use is made of several hybridization probes which are labeled, or can be labeled, differently.
29. Process according to ~~one of the preceding claims~~, characterized in that the sample is analyzed by microscopic methods.
30. Process according to ~~one of the preceding claims~~, characterized in that the analysis comprises quantitatively determining antibiotic resistances.

- ✓ 31. Use of an in-situ nucleic acid hybridization process for detecting antibiotic resistances in microorganisms.
- 5 32. Use according to Claim 31 for detecting antibiotic resistances in bacteria and protozoa.
- A 33. Use according to Claim 31 ~~or 32~~ for detecting resistances to macrolide, lincosamide, amino-
10 glycoside, aminocyclitol, tetracycline and chloramphenicol antibiotics.
34. Use according to Claim 33 for detecting resistances to macrolide antibiotics selected from
15 the group consisting of clarithromycin, erythromycin, azithromycin and roxithromycin.
- A 35. Use according to Claim 31 ~~or 32~~ for detecting resistances to aminoglycoside antibiotics selected
20 from the group consisting of streptomycin, neomycin, paromomycin, kanamycin, gentamicin, tobramycin, amikacin, netilmicin and sisomicin.
- ✓ 36. Reagent kit for typing microorganisms and/or antibiotic resistances in microorganisms by in-situ hybridization, comprising
25 (a) means for preparing the sample, and
(b) at least one hybridization probe which is specific for a nucleic acid sequence in
30 microorganisms which is associated with antibiotic resistances, and/or at least one hybridization probe which is specific for a species or genus of microorganisms.
- 35 37. Reagent kit according to Claim 36, characterized in that

the means for preparing the sample comprise a presumptive medium and, where appropriate, means for enriching microorganisms.

- 5 38. Reagent kit according to Claim 37,
characterized in that
the presumptive medium contains a nutrient
solution containing a nitrogen source and other
essential components and also, where appropriate,
10 reducing substances and/or oxygen-repelling
additives.
39. Reagent kit for typing microorganisms and/or
[lacuna] antibiotic resistances in microorganisms,
15 comprising
(a) a presumptive medium for microorganisms, and
(b) means for typing microorganisms and/or for
detecting antibiotic resistances.
- 20 40. Reagent kit according to Claim 39,
characterized in that
the presumptive medium contains a nutrient
solution containing a nitrogen source and other
essential components and also, where appropriate,
25 reducing substances and/or oxygen-repelling
additives.
- A* 41. Reagent kit according to Claim 39 ~~or 40~~,
characterized in that
30 the means for typing microorganisms comprise
indicator substances which are dissolved and/or
suspended in the presumptive medium.
42. Reagent kit according to Claim 41,
35 characterized in that.
it contains a urease indicator for detecting
Helicobacter spec., in particular H.pylori and/or
H.heilmannii.

~~43. Use of a reagent kit according to one of Claims 36 to 42 in a process according to one of Claims 1 to 30.~~

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44. Use of an oligonucleotide from a region of the V domain of the 16S rRNA for the species-specific detection of Helicobacter, in particular of H. pylori and/or H. heilmannii.

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45. Use according to Claim 44, characterized in that the oligonucleotide contains the sequence depicted in SEQ ID NO. 5, 6, 7, 8, 9, 10, 11, and/or 12, or at least a part region thereof which is 10 nucleotides in length.

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46. Oligonucleotide, characterized in that it contains the sequence depicted in SEQ ID NO. 5, 6, 7, 8, 9, 10, 11 and/or 12, or at least a part region thereof which is 10 nucleotides in length.

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47. Oligonucleotide according to Claim 46, characterized in that it carries a labeling group.

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48. Use of an oligonucleotide from a bacterial 23S rRNA for detecting antibiotic resistances.

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49. Use according to Claim 48, characterized in that the oligonucleotide contains the sequence depicted in SEQ ID NO. 1, SEQ ID NO. 2 or SEQ ID NO. 3.

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50. Use according to Claim 48 ~~or 49~~ together with a wild type-specific oligonucleotide, in particular

an oligonucleotide which contains the sequence depicted in SEQ ID NO. 4.

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- 5 51. Oligonucleotide,
characterized in that
it contains the sequence depicted in SEQ ID NO. 1,
2, 3, or 4, or at least a part region thereof
which is 10 nucleotides in length.
- 10 52. Oligonucleotide according to Claim 49,
characterized in that
it carries a labeling group.